STUDIES ON THE TRANSPARENCY OF AGAR-GEL

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By

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It is well known that agar is generally graded by determining the temperature of transition from gel to liquid, the transparency of gel, the gel strength, viscosity, ash content and content of other impurities. Among them, the transparency is one of the most important factors to discriminate the character of agar used for bacteriological culture work. It is often influenced by the content of inorganic substances and other impurities. They are removed by such laboratory procedures as vacuum filtration, electrodialysis (1), high speed centrifugation (2) or by pretreatments such as washing and soaking the raw materials with sufficient water and avoiding an excess of calcium hypochlorite used in the industrial process (3). However, the products thus obtained do not give a clear gel. It is nearly opaque in thick layers.

There have been very few papers dealing with the transparency of agar-gel. The present report describes the results of experiments conducted by the authors on this problem.

1. The wave-length for measuring clarity of sol and gel of agar.

The materials used were the agars made from Korean *Gelidium amansii* by the usual process and purified by using charcoal and celite 535. Unless otherwise stated, they were employed throughout the following experiments.

The wave-length for measuring the clarity of sol and gel of agar was determined by the following method:

1.5 grams of agar were mixed with 100 ml of distilled water and boiled until completely dissolved. Immediately after dissolving a part of the solution was put in a photometer cell and left to make gel at room temperature for exactly one hour. Then the transmittance was measured at 420 to 660 mμ wave-length by Hitachi spectrophotometer Type EPU-2A. The results are summarised in Table 1 and Fig. 1. Fig. 1 shows that the value of transmittance increased with a shift from short to long in wave-length. Although it did not show any characteristic peak

Table 1, The transmittance of sol and gel in 420 to 660 mμ of wave-length range.

Time (min.)	Transmittance (%)							
	420 mμ	460 mμ	480 mμ	500 mμ	540 mμ	580 mμ	620 mμ	660 mμ
0	83	94	96	96	98	100	100	100
3	83	92	96	96	98	100	100	100
5	82	90	96	96	98	100	100	100
8	72	82	88	88	89	93	93	94
11	61	73	80	83	86	88	89	90
14	52	65	72	73	78	84	85	87
20	44	58	65	67	78	79	82	84
30	40	55	62	64	70	77	80	83
40	38	55	60	62	69	76	79	82
50	38	53	60	62	69	76	79	82
60	38	53	60	62	69	76	79	82

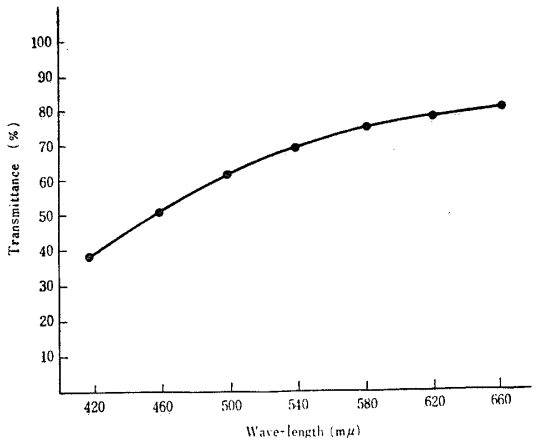


Fig. 1. The wave-length and the transmittance of agar-gel.

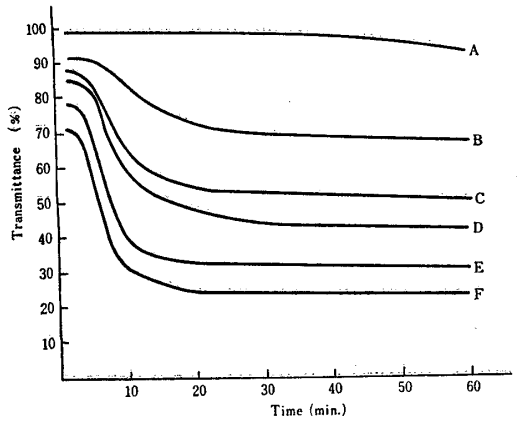
in the range of the wave-length tested, the difference between the transmittance of sol and gel was the greatest at 420mμ as seen in Table 1.

Therefore the authors employed the 420mμ wave-length for measuring the clarity of sol and gel of agar throughout the following experiments.

2. The variations of clarity during the transition from sol to gel.

i) The change of transparency in the transition time from sol to gel.

The sample agar was dissolved in hot water and the change of clarity during the transition from sol to gel was observed measuring at 420mμ by the method mentioned above. The results are presented in Fig. 2. It shows that the transparency of the agar was almost unchanged in the state of sol and then decreased with the forming of gel, especially showing a steep gradient in higher concentration. After that, it became again constant.



A: 0.1% solution of agar
B: 0.5% 〃
C: 0.8% 〃
D: 1.0% 〃
E: 1.5% 〃
F: 2.0% 〃

Fig. 2. The change of transparency in transition time from sol to gel.

ii) The relationship between the clarity and temperature of agar solution.

As shown in Fig. 3, the transparency of agar solution was directly proportional to the rise of temperature in the transition time from sol to gel but remained constant in both states of sol and gel. It is clearly seen that the dropping of transparency was dependent on the concentration and temperature of agar under the conditions employed.

When gel-formation starts, the trasparency suddenly decreases and became stable after gel formation was completed. These results agreed with those of K. Krishnamurti (4). He has already pointed out that Tyndall phenomenon of agar was more distinct in gel than in sol and its influence was greater with the lowering of temperature.

P.K. Katti (5) has studied the clarity in the course of transition from hot sol to gel and found that gel set at lower temperatures and contained larger particles in this state.

As seen in Fig. 3, the transparency decreased continuously until the gel was formed completely. After that, it remained constantly even at 10°C.

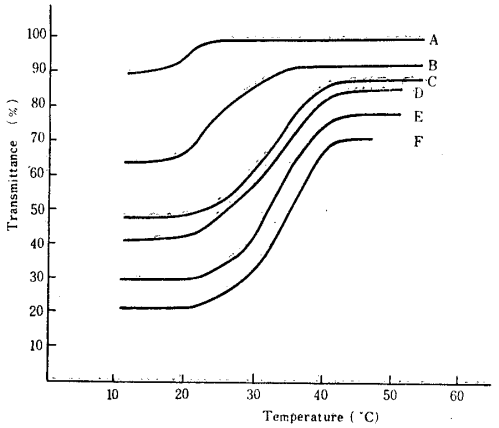


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Fig. 3. The relationship between the temperature of agar solution and transmittance.

From the fact that the clarity of sol and gel became stable, it was assumed that the agar particles so actively conducted themselves with Brownian motion that the agar was transparent in the state of sol. However, there occurred the change of agar particles in number, size and shape during the next cooling period. Then they started to aggregate and form gel. Subsequently the clarity began to decrease with the forming of gel. After reticulate structure was formed completely, it became stable and the transparency of agar-gel became also constant.

iii) The relationship between the clarity and agar concentration.

The experiments on change of clarity during sol-gel transition were carried out especially in relation to the concentration of agar. A summary of the results is indicated in Fig. 4.

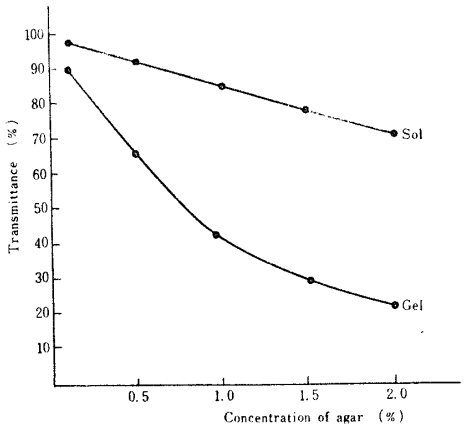

Fig. 4. The relationship between the transmittance and agar concentration.

The transparency of agar gel gradually decreased with the concentration. In the case of sol, it showed a linear relation with the concentration but its slope is slow.

Considering the transparency of sol depending on the number, size and shape of particles in the solution, it is quite natural that the clarity was directly proportional to the concentration of agar. However the fact that the clarity of agar-gel steeply decreased with the concentration is principally due to the diminution of light penetration into the gel in which the reticulate structure of gel became more dense with the increased concentration.

In the agar industry, it is very important to know the amount of agar extracted from raw materials. The colloidal titration (6, 7) and filter paper-method (8) have been used for determining this. But it is usually done by employing the full procedures of making agar or by evaporating the agar solution.

As mentioned above, there is a linear relationship between the transparency of sol and the concentration of agar. Therefore it is possible to know the amount of agar by measuring the transparency of the solution in autoclave. This is a very simple and useful method for determining the lots of samples in a short time.

3. The relationship between the clarity, gel strength and melting point of gel.

Since the gels of agars prepared from different sources generally differ from each other in transparency, we carried out the following experiments to know the relation to clarity, gel strength and melting point of gel made from *Gelidium*-, *Ceramium*- and *Gracilaria*-agar and *Hypnea* extract respectively.

The difference of transmittance between sol and gel of agar was tentatively named D. The gel strength and melting point of gel were determined by the following procedures;

3 grams of agar were dissolved completely in 200 ml of water by heating. A part of the solution was taken in 10 mm cube and the transmittance at 420 mμ by the spectrophotometer were immediately read. After that, it was left standing to make gel at room temperature for exactly one hour and the transmittance of the gel formed was read again.

The rest of the solution was put in a small can and cooled to make gel at room temperature. Then the gel strength was measured by the Nikkansui Shiki method using the Nikkansui Shiki gelometer after standing for 15 hours. The melting point of gel was estimated by Tanii's method (9).

A summary of the determination of D, gel strength and melting point of gel are shown in Table 2.

Both transparency and D had no correlation with the gel strength. It is very

Table 2. The relationship between the clarity, gel strength and melting point of agar-gel.

Materials		Transmittance of sol (%)	Transmittance of gel (%)	D*	Gel strength (g/cm²)	Melting point of gel (°C)
Gelidium agar	1	84	35	49	380	84
	2	83	35	48	420	84
	3	82	36	46	460	84
	4	81	34	47	490	85
	5	82	33	49	510	85
Ceramium agar	1	83	40	43	420	82
	2	92	49	43	450	82
	3	77	38	39	530	82
	4	73	34	39	570	83
	5	83	42	41	630	83
Gracilaria agar		72	32	40	650	83
eypnea extract	1	69	64	5	470	50
	2	64	62	2	410	50
	3	69	67	2	370	48
	4	73	70	3	360	51

*D=Transmittance of sol−Transmittance of gel

interesting that the agar, which is low in melting point, such as *Hypnea*-agar, had a small D-value and gave a clear gel.

It may be considered that *Hypnea* extract is different from *Gelidium*- and *Gracilaria*-agar in the size, number and shape of particles and bonds of water. *Hypnea* extract (10) seems to be very similar to κ-carrageenin composed of D-galactose, 3,6-anhydro-D-galactose and sulfate groups. In order to form gel, electrolyte as potassium chloride is added to the aqueous extract. As the gel formed contains much water and is hydrated, it is low in gel strength and melting point but high in transparency.

On the other hand, *Gelidium*-, *Gracilaria*- and *Ceramium*-agar mainly composed of a neutral polymer of galactose (11) are able to form gel without any electrolyte. There occurs the aggregation of particles of agar in the course of transition from sol to gel. Thus the D-value defined as the difference of transparency between sol and gel, will be one of the indices indicating the size, number and shape of the aggregating particles.

This assumption may be proved by the following experiments. Namely, the pH of sol of *Gelidium* agar was decreased by addition of dilute hydrochloric acid and the transparency and gel strength of agar-gel concerned were measured by the method mentioned above. The results are summarized in Table 3. It shows that

Table 3. The relationship between pH, clarity and gel strength.

pH	Transmittance of sol (%)	Transmittance of gel (%)	D*	Gel strength (g/cm^2)
6.8	84.0	35.5	48.5	450
4.7	84.0	26.5	57.5	330
3.0	84.0	11.0	73.0	below 100
2.0	84.0	84.0	0	sol

D*=Transmittance of sol − Transmittance of gel

the transparency was increased inversely to the falling pH until pH 2. It was also seen that the gel acidulated decreased in gel strength. These facts mean that the gel thus treated was lightly loosened in the linkage and resulted an increase in number of particles. Below pH 2, the transparency became the same as sol because the agar molecules concerned greatly decreased the ability to form gel and did not aggregate any longer.

Summary

1) The value of transmittance of agar gel increased with a shift from short to long in the range of 420 to 620mμ of wave-length.

2) The transparency of agar was almost unchanged in the state of sol but

decreased with gel formation and became again constant after the gel formation was completed.

3) The transparency of agar sol depended on the number, size and shape of particles in the solution and was proportional to the concentration of agar.

4) Both the transparency and D defined as the difference of transparency between sol and gel had no correlation with the gel strength.

5) The D-value will show the size, shape and number of the aggregating particles.

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